

Coagulation Activity Is Increased in the Left Atrium of Patients With Mitral Stenosis

KEIJI YAMAMOTO, MD, UICHI IKEDA, MD, YOSHITANE SEINO, MD, HIDEAKI MITO, MD, HIDEYUKI FUJIKAWA, MD, HIROMICHI SEKIGUCHI, MD, KAZUYUKI SHIMADA, MD

Tochigi, Japan

Objectives. This study investigated the hemostatic status of the right and left atria in patients with mitral stenosis.

Background. Systemic thromboembolism is a serious major complication in patients with mitral stenosis. However, the pathogenesis of thromboembolism in mitral stenosis is not fully understood.

Methods. We determined the plasma levels of biochemical markers for platelet activity (platelet factor 4 and beta-thromboglobulin) and status of thrombin generation (fibrinopeptide A and thrombin-antithrombin III complex) and fibrinolysis (D-dimer and plasmin- α_2 -plasmin inhibitor complex) in specimens of blood obtained from the peripheral vein and right and left atria of 12 consecutive patients with mitral stenosis who were undergoing percutaneous mitral valvuloplasty.

Results. Plasma levels of platelet factor 4, beta-thromboglobulin, D-dimer and plasmin- α_2 -plasmin inhibitor complex in the patients did not differ significantly between the right and left atria, whereas levels of fibrinopeptide A and thrombin-antithrombin III

complex in the left atrium were significantly higher than those in the right atrium (fibrinopeptide A in the left and right atria 19.35 ± 4.64 and 6.31 ± 0.75 ng/ml [mean \pm SE], respectively, $p < 0.02$; thrombin-antithrombin III complex in the left and right atria 11.45 ± 2.29 and 3.98 ± 0.60 ng/ml, respectively, $p < 0.01$). Levels of fibrinopeptide A and thrombin-antithrombin III complex in the left atrium did not correlate with mean transmitral gradient, dimension of the left atrium or reciprocal of the mitral valve area. Peripheral blood plasma levels of von Willebrand factor antigen were significantly higher in the patients than in an age-matched control group of normal subjects ($168 \pm 25\%$ and $99 \pm 7\%$, respectively, $p < 0.05$) but showed no difference in the peripheral blood and right and left atria of the patients.

Conclusions. The coagulation system is activated in the left atrium of patients with mitral stenosis even during anticoagulation.

(*J Am Coll Cardiol* 1995;25:107-12)

Systemic thromboembolism is a serious complication in patients with valvular heart disease, and its incidence is highest in those with mitral stenosis (1-7). The incidence of embolism in large groups of such patients is $\sim 4\%$ /year (8). The Framingham Study (9) reported an 18-fold increase in the incidence of stroke in patients who had mitral stenosis and atrial fibrillation compared with matched control subjects. Thus, the prophylactic administration of anticoagulant agents appears to be indicated in selected patients with mitral stenosis, especially those with atrial fibrillation (10). However, the efficacy of platelet antiaggregant agents, such as aspirin, has not been convincingly established.

Sensitive new biochemical markers for assessing platelet activity and the status of thrombin generation and fibrinolysis have made it possible to evaluate such activities in patients with valvular heart disease, ischemic heart disease, deep vein thrombosis and pulmonary embolism (11-20). It has been

reported that plasma levels of D-dimer and thrombin-antithrombin III complex (12) and von Willebrand factor antigen (15) are significantly elevated in patients with mitral stenosis. However, these studies evaluated hemostatic status only at the peripheral level and evaluated blood obtained from the peripheral vein. In the present study, we investigated plasma levels of biochemical markers for platelet activity and the status of thrombin generation and fibrinolysis in the peripheral vein and right and left atria of 12 consecutive patients with rheumatic mitral stenosis who were undergoing percutaneous mitral valvuloplasty. We believe that this is the first report to discuss the hemostatic status of the atria in such patients.

Methods

Clinical characteristics. Between March 1992 and March 1993, 12 patients (2 men, 10 women; mean \pm SE age 53 ± 2 years, range 39 to 68) with severe rheumatic mitral stenosis underwent percutaneous mitral valvuloplasty by the antegrade transseptal approach using an Inoue balloon (21). Their clinical characteristics are summarized in Table 1. Eleven patients had chronic atrial fibrillation, and one had sinus rhythm before valvuloplasty. All 11 patients with atrial fibrillation had re-

From the Department of Cardiology, Jichi Medical School, Minamikawachi, Tochigi, Japan. This study was supported in part by Grants 5670632 and 5770477 from the Ministry of Education, Culture and Science, Tokyo, Japan.

Manuscript received December 31, 1993; revised manuscript received May 26, 1994, accepted August 25, 1994.

Address for correspondence: Dr. Uichi Ikeda, Department of Cardiology, Jichi Medical School, Minamikawachi-Machi, Tochigi 329-04, Japan.

Table 1. Clinical Characteristics of 12 Patients Who Underwent Percutaneous Mitral Valvuloplasty

Gender (M/F)	2/10
Age (yr)	53 ± 2
History of rheumatic fever	5
Previous surgical commissurotomy	1
Previous PMV	1
NYHA functional class	
II	9
III	3
Cardiac rhythm	
Sinus	1
Atrial fibrillation	11
Oral anticoagulants	11
INR	2.7 ± 0.1
Mitral calcification (fluoroscopy)	10
Mean left atrial diameter (mm)	59 ± 2
Echocardiographic score >8	7
Mitral regurgitation	
None	5
1/4	4
3/4	3
Mean transmitral gradient (mm Hg)	13.6 ± 1.6
Mitral valve area (cm ²)	0.81 ± 0.07

Data presented are mean value ± SE or number of patients. F = female; INR = international normalized ratio; M = male; NYHA = New York Heart Association; PMV = percutaneous mitral valvuloplasty.

ceived warfarin therapy for at least 3 months (international normalized ratio 2.0 to 4.0, mean [±SE] 2.7 ± 0.1) but no antiplatelet drugs or hemorrhheologically active agents, such as analgesic agents and estrogen-containing drugs. According to the practice of our cardiac catheterization laboratory, warfarin administration was stopped on the day of valvuloplasty. Nine patients were in New York Heart Association functional class II and three in class III. One patient had previously undergone commissurotomy, and one had a previous percutaneous mitral valvuloplasty. Criteria for exclusion from valvuloplasty were significant mitral regurgitation, aortic stenosis, aortic regurgitation, history of systemic embolism, left atrial clot (as detected by two-dimensional transesophageal echocardiography with a biplane probe), diabetes mellitus and renal or hepatic insufficiency. An age-matched control group of 15 normal volunteers (3 men, 12 women; mean age 53 ± 2 years, range 35 to 66) received no medications. The study was approved by our institutional human investigations committee, and written informed consent was obtained from all patients and volunteers before the study.

All patients underwent M-mode, two-dimensional and Doppler echocardiography on the day before valvuloplasty. Mean (±SE) left atrial diameter determined by M-mode echocardiography (22) was 59 ± 2 mm (Table 1). Transesophageal echocardiography performed in all patients detected no intracardiac thrombus. Morphologic characteristics of the mitral valve and subvalvular structures were classified using an echocardiographic scoring system (23).

Cardiac catheterization and valvuloplasty. Right and left heart studies, including measurements of cardiac output, bi-

plane left ventriculography and right atriography, and coronary angiography were performed by the percutaneous technique from the left groin immediately before percutaneous mitral valvuloplasty. This procedure was performed with an Inoue single-balloon catheter (17). In brief, the balloon catheter was inserted into the left ventricle transseptally according to the Brockenbrough technique. After its distal half was inflated at the left ventricle, the balloon was pulled back to the mitral orifice. It was then fully inflated until its indentation had disappeared. It was then pulled back to the left atrium and deflated. The diameter of the inflated balloon was 24 mm in five patients, 26 mm in six patients and 28 mm in one patient. These manipulations were performed under fluoroscopy with the same predetermined projections as those used in left ventriculography. Effective areas of balloon dilation were determined by geometric analysis and normalized for body surface area, as described previously (24). The severity of mitral regurgitation was assessed according to the method of Sellers et al. (25). Pressures in the left atrium and left ventricle were simultaneously recorded to determine the mean transmitral gradient (13.6 ± 1.6 mm Hg, mean ± SE) before valvuloplasty (Table 1). Cardiac output was determined by thermodilution in 11 patients and by the direct Fick method in one with moderate tricuspid regurgitation. Mitral valve area calculated by the Gorlin formula (26) was 0.81 ± 0.07 cm² (mean ± SE) before valvuloplasty.

Blood sampling. Samples of the peripheral venous blood were obtained for measurement of molecular markers at 9 AM before percutaneous mitral valvuloplasty to exclude the possible influence of circadian variations (27-29). Immediately before the administration of heparin and inflation of balloon during valvuloplasty, blood samples were obtained from the left and right atria through a thermodilution catheter (7F) and an Inoue balloon catheter (12F), respectively. A trained physician carried out blood sampling using the two-syringe technique. A preliminary study showed no difference in the levels of biochemical markers in samples obtained from the same sites through the two types of catheters, and drawing blood samples from these catheters did not cause an artificial increase in the levels of biochemical markers. Nine parts of whole blood were mixed with one part of 3.8% sodium citrate solution for assay of thrombin-antithrombin III complex, plasmin- α_2 -plasmin inhibitor complex and von Willebrand factor antigen. Four parts of whole blood were gently mixed with one part of a solution containing sodium citrate, theophylline, adenosine and dipyridamole for assay of platelet factor 4 and beta-thromboglobulin (30). A volume of 1.6 ml of whole blood was drawn into a plastic tube containing 200 U of heparin and 4,000 kIU of aprotinin for assay of fibrinopeptide A and D-dimer. Plasma was separated by centrifugation at 2,000 g for 30 min and stored at -80°C until the molecular markers were assayed.

Plasma assays. Enzyme-linked immunosorbent assay (ELISA) kits (Diagnostica Stago, Asnières, France) were used for quantitative determination of platelet factor 4 and beta-thromboglobulin (31). Levels of fibrinopeptide A, thrombin-

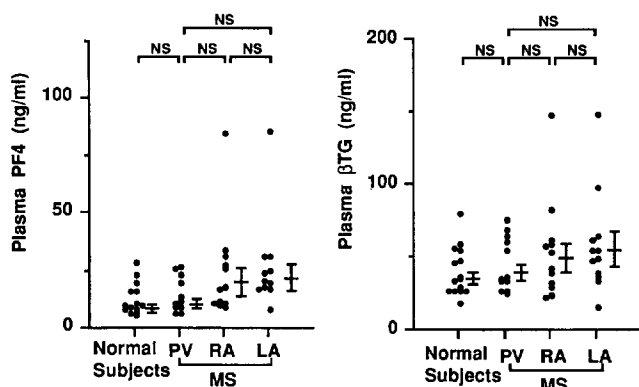


Figure 1. Scatterplots showing plasma levels of platelet factor 4 (PF4) and beta-thromboglobulin (βTG) in normal subjects (n = 15) and in the peripheral blood and right (RA) and left (LA) atria of patients with mitral stenosis (MS) (n = 12). PV = peripheral vein. Data are mean value ± SE.

antithrombin III complex, D-dimer and plasmin-α₂-plasmin inhibitor complex were also determined with ELISA kits (Diagnostica Stago; Behringwerke, Marburg, Germany; Agen Biomedical, Brisbane, Australia; and Teijin, Tokyo, Japan, respectively) (32-35). Concentrations of von Willebrand factor antigen were determined by ELISA (Diagnostica Stago) (36), with values expressed as a percent of that obtained in pooled normal plasma.

Statistical analysis. Data are expressed as mean value ± SE. Differences were analyzed by one-way analysis of variance combined with the Scheffé test. Simple linear regression analysis was used to compare measurements obtained by means of catheterization or ultrasound as well as the levels of molecular markers for platelet activity and status of thrombin generation and fibrinolysis; *p* < 0.05 was considered statistically significant.

Results

Platelet activity. Figure 1 and Table 2 show the plasma levels of platelet factor 4 and beta-thromboglobulin used to reflect platelet activity. Levels of platelet factor 4 in the

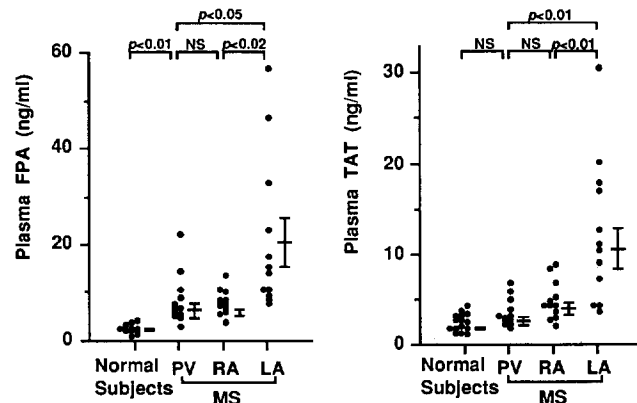


Figure 2. Scatterplots showing plasma levels of fibrinopeptide A (FPA) and thrombin-antithrombin III complex (TAT) in normal subjects (n = 15) and in the peripheral blood and right (RA) and left (LA) atria of patients with mitral stenosis (MS) (n = 12). In patients with mitral stenosis, peripheral blood plasma fibrinopeptide A levels were significantly higher than those in normal subjects, and plasma fibrinopeptide A and thrombin-antithrombin III complex levels in the left atrium were significantly higher than those in the peripheral blood and right atrium. PV = peripheral vein. Data are mean value ± SE.

peripheral blood did not differ significantly between patients with mitral stenosis and normal subjects. Levels of platelet factor 4 in the patients did not differ significantly with respect to the peripheral blood and right and left atria. Plasma levels of beta-thromboglobulin also did not differ significantly between the patients and normal subjects or the peripheral blood and right and left atria in the patients.

Status of thrombin generation. Plasma levels of fibrinopeptide A and thrombin-antithrombin III complex used as a marker of thrombin activity, are shown in Figures 2 and 3 and Table 2. Peripheral blood levels of fibrinopeptide A in the patients with mitral stenosis significantly exceeded those in the normal subjects. Levels of fibrinopeptide A were significantly higher in the left atrium than in the peripheral blood or right atrium of the patients. Plasma levels of thrombin-antithrombin III complex in the peripheral blood did not differ significantly

Table 2. Biochemical Markers in Normal Subjects and Patients With Mitral Stenosis

	Normal Subjects (n = 15)	Patients With Mitral Stenosis (n = 12)		
		PV	RA	LA
PF4 (ng/ml)	8.5 ± 1.8	10.8 ± 2.1	20.3 ± 6.1	22.3 ± 5.7
βTG (ng/ml)	34.3 ± 4.2	39.1 ± 5.3	48.4 ± 9.9	55.1 ± 12.2
FPA (ng/ml)	1.08 ± 0.19	6.73 ± 1.53*	6.31 ± 0.75	19.35 ± 4.64†‡
TAT (ng/ml)	1.75 ± 0.19	2.63 ± 0.44	3.98 ± 0.60	11.45 ± 2.29§
DD (ng/ml)	55.4 ± 5.9	67.9 ± 10.2	63.8 ± 7.6	63.3 ± 7.1
PIC (μg/ml)	0.55 ± 0.04	0.69 ± 0.08	0.68 ± 0.07	0.69 ± 0.07
vWF:Ag (%)	99 ± 7	168 ± 25¶	150 ± 23	151 ± 23

**p* < 0.01, ¶*p* < 0.05 versus normal subjects. †*p* < 0.05, §*p* < 0.01 versus peripheral vein (PV). ‡*p* < 0.02, ||*p* < 0.01 versus right atrium (RA). Data presented are mean value ± SE. DD = D-dimer; FPA = fibrinopeptide A; LA = left atrium; PF4 = platelet factor 4; PIC = plasmin-α₂-plasmin inhibitor complex; TAT = thrombin-antithrombin III complex; vWF:Ag = von Willebrand factor antigen; βTG = beta-thromboglobulin.

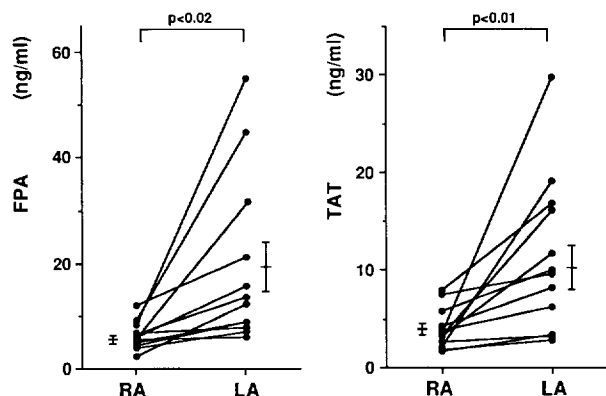


Figure 3. Plasma levels of fibrinopeptide A (FPA) and thrombin-antithrombin III complex (TAT) in the right (RA) and left (LA) atria of 12 patients with mitral stenosis. Data are mean value \pm SE.

between patients and normal subjects. However, in the left atrium of the patients, these levels significantly exceeded those in the peripheral blood or the right atrium. There was a significantly positive relation between the plasma levels of fibrinopeptide A and thrombin-antithrombin III complex in the left atrium of the patients (Fig. 4).

Fibrinolytic status. Plasma levels of D-dimer and plasmin- α_2 -plasmin inhibitor complex, which reflect active fibrinolysis, are shown in Figure 5 and Table 2. Levels of these molecular markers in the peripheral blood did not differ significantly between patients and normal subjects. Plasma levels of these molecular markers also did not differ significantly in the peripheral blood and right and left atria of the patients.

Levels of von Willebrand factor. Levels of von Willebrand factor antigen in the peripheral blood were significantly higher in the patients than in the normal subjects but did not differ significantly in the peripheral blood and right and left atria of the patients (Fig. 6, Table 2).

Relation between biochemical markers and hemodynamic variables. Of the several biochemical markers evaluated in the left atrium of patients with mitral stenosis, fibrinopeptide A and

Figure 4. Relation between plasma levels of fibrinopeptide A (FPA) and thrombin-antithrombin III complex (TAT) in the left atrium (LA) of patients with mitral stenosis. Regression analysis revealed a highly significant relation between the two molecular markers ($y = 0.42x + 3.36$, $r = 0.85$, $p = 0.002$).

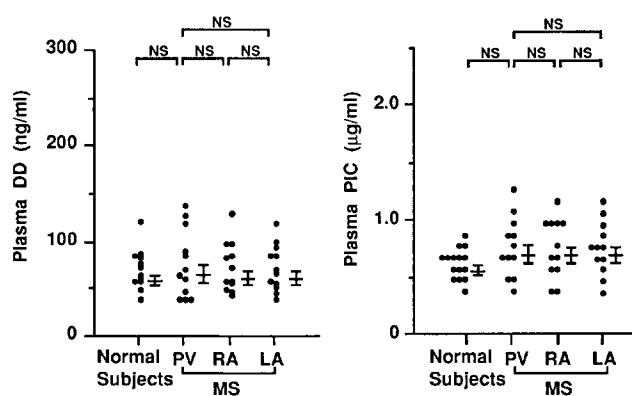
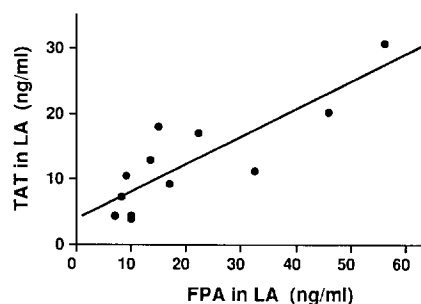


Figure 5. Scatterplots showing plasma levels of D-dimer (DD) and plasmin- α_2 -plasmin inhibitor complex (PIC) in normal subjects ($n = 15$) and in the peripheral blood and right (RA) and left (LA) atria of patients with mitral stenosis (MS) ($n = 12$). PV = peripheral vein. Data are mean value \pm SE.

thrombin-antithrombin III complex levels were shown to be significantly increased. We then analyzed the correlation between the levels of these coagulation markers and hemodynamic variables. Plasma levels of fibrinopeptide A and thrombin-antithrombin III complex in the left atrium of the patients did not correlate well with the diameter of the left atrium, mean trans-mitral gradient or reciprocal mitral valve area (Table 3).

Discussion

Thrombin specifically cleaves fibrinopeptide A from the amino termini of the fibrinogen α chain and initiates fibrin generation (37). Thrombin is very rapidly bound and thereby inactivated by its main physiologic inhibitor, antithrombin III, producing thrombin-antithrombin III complex. Plasma fibrinopeptide A and thrombin-antithrombin III complex levels represent a very sensitive marker for thrombin activity in vivo; direct measurement of free thrombin is impossible because of

Figure 6. Scatterplots showing plasma levels of von Willebrand factor antigen (vWF:Ag) in normal subjects ($n = 15$) and in the peripheral blood and right (RA) and left (LA) atria of patients with mitral stenosis (MS) ($n = 12$). Peripheral plasma levels of von Willebrand factor antigen in patients with mitral stenosis significantly exceeded those determined in normal subjects. PV = peripheral vein. Data are mean value \pm SE.

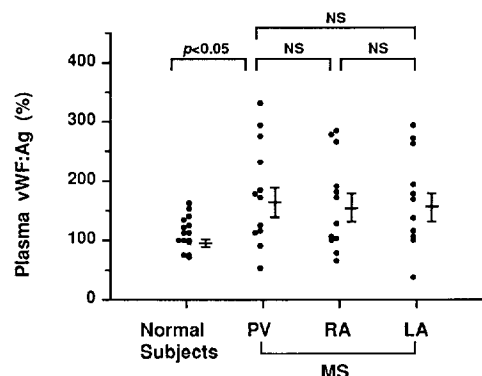


Table 3. Correlation Between Biochemical Markers in Left Atrium and Clinical or Hemodynamic Variables in Patients With Mitral Stenosis

	FPA (ng/ml)		TAT (ng/ml)	
	r Value	p Value	r Value	p Value
Left atrial diameter (mm)	0.05	0.88	0.11	0.73
Mean transmitral gradient (mm Hg)	0.42	0.17	0.26	0.41
1/mitral valve area (cm ⁻²)	0.40	0.20	0.45	0.14

FPA = fibrinopeptide A; TAT = thrombin-antithrombin III complex.

its very short half-life. In this study, plasma levels of fibrinopeptide A and thrombin-antithrombin III complex in the left atrium of patients with mitral stenosis significantly exceeded those in the peripheral blood or the right atrium, demonstrating a state of increased coagulability in the left atrium of these patients even during anticoagulation.

Hemostatic condition in mitral stenosis. The status of thrombin generation and fibrinolysis in the peripheral blood of patients with mitral stenosis has been studied by several groups (38). Yasaka et al. (12) reported that levels of fibrinopeptide A, fibrinopeptide B-beta-15-42, D-dimer and antithrombin III in the peripheral blood were significantly higher in patients with mitral stenosis than in normal subjects. Jafri et al. (13) reported that patients with mitral stenosis who were in sinus rhythm showed a significant increase in D-dimer levels in the peripheral blood over those in control subjects, suggesting that the coagulation system is activated in patients with mitral stenosis. In our study, all but one patient had chronic atrial fibrillation. Thus, chronic atrial fibrillation may partly contribute to the elevated fibrinopeptide A and thrombin-antithrombin III complex levels seen in the left atrium. However, levels of fibrinopeptide A and thrombin-antithrombin III complex levels in the right atrium were not elevated in 11 patients with atrial fibrillation compared with levels in the peripheral blood (Fig. 2), suggesting that the presence of atrial fibrillation itself does not cause an increase in fibrinopeptide A and thrombin-antithrombin III complex levels in the left as well as the right atrium.

A shortened platelet survival time has been documented in patients with mitral stenosis, suggesting that they have activated platelet activity (39). Fukuda and Nakamura (40) reported that platelet function was increased in all patients with rheumatic valvular disease on the basis of findings of high plasma beta-thromboglobulin values. In our study, plasma levels of platelet factor 4 and beta-thromboglobulin in the peripheral blood did not differ significantly between patients and normal control subjects. Levels of platelet factor 4 and beta-thromboglobulin tended to increase in each atrium compared with levels in the peripheral blood, but not to a statistically significant extent. We speculate that platelet activity is not increased within the cardiac chambers of patients with mitral stenosis. However, the possibility that platelet function may have been suppressed by warfarin administration cannot be ruled out (41).

In our study, severity of stenosis on the basis of mitral valve area was not correlated with levels of fibrinopeptide A and

thrombin-antithrombin III complex in the left atrium. A spontaneous left atrial echo contrast is often observed in patients with a history of arterial embolization or left atrial thrombi and is associated with conditions that favor stasis of blood in the left atrium in mitral stenosis (42,43). Rheologic abnormalities in the left atrium of patients with mitral stenosis may contribute to the hypercoagulable status. However, the incidence of thromboembolism and the size of the mitral orifice do not show a simple correlation (44). In this study, we observed spontaneous echo contrast in the left atrium of some patients, but there was no relation between its presence and levels of fibrinopeptide A and thrombin-antithrombin III complex in the left atrium (data not shown).

The large multimeric glycoprotein, von Willebrand factor, is found in endothelial cells, plasma and platelet alpha granules and mediates platelet adhesion to the vascular subendothelium. This protein is essential for the formation of platelet thrombi in flow that is characterized by high shear stress (45). Penny et al. (15) reported that the von Willebrand factor antigen concentration in the peripheral blood was significantly higher in patients with mitral stenosis and that this elevation was associated with a significant increase in pulmonary vascular resistance. These investigators speculated that there was increased synthesis of von Willebrand factor in the pulmonary circulation in these patients. In the present study, levels of von Willebrand factor antigen in the peripheral blood of patients with mitral stenosis were significantly higher than those in age-matched normal subjects. However, we observed no correlation between levels of von Willebrand factor antigen and pulmonary vascular resistance ($r = 0.09$, $p = 0.54$). Patients with mitral stenosis showed no significant differences in von Willebrand factor antigen levels in peripheral blood and right and left atria. Although elevation of von Willebrand factor may contribute to the thrombotic tendency seen in patients with mitral stenosis, our study could not clarify its site of production. The therapeutic benefit directed at von Willebrand factor in these patients also remains to be clarified.

Adams et al. (46) evaluated 84 patients with mitral stenosis followed up for 20 years and found that patients without anticoagulation had a higher mortality rate than those with anticoagulation. However, there have been no prospective randomized trials to evaluate the efficacy of anticoagulation in the primary prevention of systemic embolism in mitral stenosis. Anticoagulation is generally recommended for treating patients with mitral stenosis, particularly those with atrial fibrillation, a large left atrium or heart failure or patients with a history of systemic embolism (47). Asakura et al. (14) demonstrated that patients with mitral stenosis and atrial fibrillation showed a highly hypercoagulable status. In vivo activation of blood coagulation was more effectively controlled in those patients who received warfarin than in those taking aspirin. However, the incidence of thromboembolism still can not be disregarded with those medications.

Limitation of the study. We cannot rule out completely the effect of puncture of the atrial septum itself on coagulation activity in both atria; needle perforation of the septum and

catheter dilation of the septal puncture could cause raw edges of the septal perforation to be exposed to atrial blood. However, the lack of difference in platelet factor 4 and beta-thromboglobulin levels between the left and right atria is somewhat indicative that the septal wound is not a fatal flaw in this study.

Conclusions. We believe that our study demonstrates for the first time that coagulation activity is significantly increased in the left atrium of patients with mitral stenosis even during anticoagulation, which might contribute to the pathogenesis of thromboembolism in mitral stenosis. Our findings also suggest that platelet activity is not significantly increased in the left atrium of these patients.

References

- Selzer A, Cohn KE. Natural history of mitral stenosis: a review. *Circulation* 1972;45:878-90.
- Toy JL, Lederer DA, Tulpule AT, Tandon AP, Taylor SH, McNicol GP. Coagulation studies in rheumatic heart disease. *Br Heart J* 1980;43:301-5.
- Coulshed N, Epstein EJ, McKendrick CS, Galloway RN, Walker E. Systemic embolism in mitral valve disease. *Br Heart J* 1970;32:26-34.
- Szekely P. Systemic embolism and anticoagulant prophylaxis in rheumatic heart disease. *Br Med J* 1964;1:1209-12.
- Rowe JC, Blant EF, Sprague HB, White PD. The course of mitral stenosis without surgery. *Ann Intern Med* 1960;52:741-9.
- Cerebral Embolism Task Force. Cardiogenic brain embolism. *Arch Neurol* 1986;43:71-84.
- Askey JM, Bernstein S. The management of rheumatic heart disease in relation to systemic arterial embolism. *Prog Cardiovasc Dis* 1960;3:220-32.
- Fleming HA, Bailey SM. Mitral valve disease, systemic embolism and anticoagulants. *Postgrad Med J* 1971;47:599-604.
- Wolf PA, Dawber TR, Thomas HE Jr, Kannel WB. Epidemiologic assessment of chronic atrial fibrillation and risk of stroke: the Framingham study. *Neurology* 1978;28:973-7.
- Levine HJ. Which atrial fibrillation patients should be on chronic anticoagulation? *J Cardiovasc Med* 1981;6:483-7.
- Ludlam CA, Moore S, Bolton AE, Pepper DS, Cash JD. The release of a human platelet-specific protein measured by radioimmunoassay. *Thromb Res* 1975;6:543-8.
- Yasaka M, Miyatake K, Mitani M, et al. Intracardiac mobile thrombus and D-dimer fragment of fibrin in patients with mitral stenosis. *Br Heart J* 1991;66:22-5.
- Jafri SM, Caceres L, Rosman HS, et al. Activation of the coagulation system in woman with mitral stenosis and sinus rhythm. *Am J Cardiol* 1992;70:1217-9.
- Asakura H, Hifumi S, Jokaji H, et al. Prothrombin fragment F1+2 and thrombin-antithrombin III complex are useful markers of the hypercoagulable state in atrial fibrillation. *Blood Coagulation and Fibrinolysis* 1992;3:469-73.
- Penny WF, Weinstein M, Salzman EW, Ware JA. Correlation of circulating von Willebrand factor levels with cardiovascular hemodynamics. *Circulation* 1991;83:1630-6.
- Johnsson H, Orinius E, Paul C. Fibrinopeptide A (FPA) in patients with acute myocardial infarction. *Thromb Res* 1979;16:255-60.
- Huber K, Resch I, Stefanelli T, et al. Plasminogen activator inhibitor-1 levels in patients with chronic angina pectoris with or without angiographic evidence of coronary sclerosis. *Thromb Haemostas* 1990;63:336-9.
- Sakata K, Hoshino T, Yoshida H, et al. Circadian fluctuations of tissue plasminogen activator antigen and plasminogen activator inhibitor-1 antigens in vasospastic angina. *Am Heart J* 1992;124:854-60.
- Bounameaux H, Schneider PA, Reber G, de Moerloose P, Krahenbuhl B. Measurement of plasma D-dimer for diagnosis of deep venous thrombosis. *Am J Clin Pathol* 1989;91:82-5.
- Bounameaux H, Cirafici P, de Moerloose P, et al. Measurement of D-dimer in plasma as diagnostic aid in suspected pulmonary embolism. *Lancet* 1991;337:196-200.
- Inoue K, Owaki T, Nakamura T, Kitamura F, Miyamoto N. Clinical application of transvenous mitral commissurotomy by a new balloon catheter. *J Thorac Cardiovasc Surg* 1984;87:394-402.
- Sahn DJ, De Maria A, Kisslo J, Weyman A. Recommendations regarding quantitation in M-mode echocardiography: results of a survey of echocardiographic measurements. *Circulation* 1978;58:1072-83.
- Wilkins GT, Weyman AE, Abascal VM, Block PC, Palacios IF. Percutaneous mitral valvotomy: an analysis of echocardiographic variables related to outcome and the mechanism of dilatation. *Br Heart J* 1988;60:299-308.
- Herrmann HC, Wilkins GT, Abascal VM, Weyman AE, Block PC, Palacios IF. Percutaneous balloon mitral valvotomy for patients with mitral stenosis: analysis of factors influencing early results. *J Thorac Cardiovasc Surg* 1988;96:33-8.
- Sellers RD, Levy MJ, Amplatz K, Lillehei CW. Left retrograde cardiography in acquired cardiac disease: technic, indications and interpretations in 700 cases. *Am J Cardiol* 1964;14:437-47.
- Cohen MV, Gorlin R. Modified orifice equation for the calculation of mitral valve area. *Am Heart J* 1972;84:839-40.
- Huber K, Resch I, Rose D, Schuster E, Glogar DH, Binder BR. Circadian variations of plasminogen activator inhibitor and tissue plasminogen activator levels in plasma of patients with unstable coronary artery disease and acute myocardial infarction. *Thromb Haemostas* 1988;60:372-6.
- Kluft C, Jie AFH, Rijken DC, Verheijen JH. Daytime fluctuations in blood of tissue-type plasminogen activator (t-PA) and its fast-acting inhibitor (PAI-1). *Thromb Haemostas* 1988;59:329-32.
- Angleton P, Chandler WL, Schmer G. Diurnal variation of tissue-type plasminogen activator and its rapid inhibitor (PAI-1). *Circulation* 1989;79:101-6.
- Contant G, Gouault-Heilmann M, Martnoli JL. Heparin inactivation during blood storage: its prevention by blood collection in citric acid, theophylline, adenosine, dipyridamole—C.T.A.D. mixture. *Thromb Res* 1983;31:365-74.
- Takahashi H, Yoshino N, Hanano M, Shibata A. Measurement of platelet factor 4 and β -thromboglobulin by an enzyme-linked immunosorbent assay. *Blood Vessel* 1987;18:326-35.
- Amiral J, Jeanine M, Walenga BS, Fareed J. Development and performance characteristics of a competitive enzyme immunoassay for fibrinopeptide A. *Semin Thromb Hemostas* 1984;10:228-42.
- Hoek JA, Sturk A, ten Cate JW, Lamping RJ, Berends F, Borm JJJ. Laboratory and clinical evaluation of an assay of thrombin-antithrombin III complexes in plasma. *Clin Chem* 1988;34:2058-62.
- Rylatt DB, Blake AS, Cottis LE, et al. An immunoassay for human D dimer using monoclonal antibodies. *Thromb Res* 1983;31:767-78.
- Mimuro J, Koike Y, Sumi Y, Aoki N. Monoclonal antibodies to discrete regions in α_2 -plasmin inhibitor. *Blood* 1987;69:446-53.
- Cejka J. Performance characteristics of a commercial kit for assay of factor VIII-related antigen. *Clin Chem* 1984;30:814-5.
- Bettelheim FR. The clotting of fibrinogen: II. Fractionation of peptide material liberated. *Biochim Biophys Acta* 1956;19:121-9.
- Cerebral Embolism Task Force. Cardiogenic brain embolism. *Arch Neurol* 1986;43:71-84.
- Steele PP, Weilly HS, Davies H, Genton E. Platelet survival in patients with rheumatic heart disease. *N Engl J Med* 1974;290:537-9.
- Fukuda Y, Nakamura K. The incidence of thromboembolism and the hemocoagulative background in patients with rheumatic heart disease. *Jpn Circ J* 1984;48:59-66.
- Kovacs IB, Murphy M, Barin E, Rees GM. Effect of warfarin on arterial thrombogenesis: problems of monitoring. *Am J Hematol* 1993;42:322-7.
- Daniel WG, Nellessen U, Schroder E, et al. Left atrial spontaneous echo contrast in mitral valve disease: an indicator for an increased thromboembolic risk. *J Am Coll Cardiol* 1988;11:1204-11.
- Black IW, Hopkins AP, Lee LCL, Walsh WF. Left atrial spontaneous echo contrast: a clinical and echocardiographic analysis. *J Am Coll Cardiol* 1991;18:398-404.
- Braunwald E. Valvular heart disease. In: Braunwald E, editor. *Heart Disease*. 3rd ed. Philadelphia: Saunders, 1988:1027.
- Moake JL, Turner NA, Stathopoulos NA, Nolasco LH, Hellums JD. Involvement of large plasma von Willebrand factor multimers and unusually large vWF forms derived from endothelial cells in shear stress: induced platelet aggregation. *J Clin Invest* 1986;78:1456-61.
- Adams GF, Merrett JD, Hutchison WM, Pollock AM. Cerebral embolism and mitral stenosis: survival with and without anticoagulants. *J Neurol Neurosurg Psychiatry* 1974;37:378-83.
- Chesebro JH, Adams PC, Fuster V. Antithrombotic therapy in patients with valvular heart disease and prosthetic heart valves. *J Am Coll Cardiol* 1986;8:41B-56B.